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Vibrational spectroscopy of LDLL-mixtures of amino acids

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Abstract

In equimolar mixtures of L- and DL-proline, the vibrational spectra of the LDLL-mixture is not an average between that of the L- and the DL-isomers. Instead the DL-mixture is clearly intermediate between the L- and the LDLL-spectra. Adding L-isomer to the DL-isomer, the solid state spectra becomes less similar to that of the L-isomer. Replacing respectively the LL-half and the DL-half with a second amino acid, each set can significantly alter the conformation of proline and/or the second amino acid, even though both sets (LDLL- and LLDL-mixtures) have identical structural composition. Large changes in spectral properties between LDLL- and LLDL-amino acids are consistent with diastereoismeric interactions between isomers. Infrared frequencies for chiral structures in an achiral localized environment are not predictive of the frequencies for the same chemical structures in a chiral environment.

Keywords: Infrared spectrometry; Nuclear magnetic resonance spectrometry; Amino acids; Chirality; Proline; Solid state

Although the vibrational spectra and physical properties of enantiomers are identical, those for diastereoisomers most frequently are not [1]. Interactions based on different thermodynamic properties of solids between L-isomers and equimolar mixtures of D- and L-isomers have been reviewed [2]. Chiral centers on molecular surfaces of solids have also been proposed as nucleation sites [3]. Recent nuclear magnetic resonance (NMR) research on mixtures of enantiomers of amino acids found unexpectedly large spectral differences at a 3:1 mole ratio of L:D compared to both the DL- (or *RS* or [+]/[−]) mixture and the pure L-isomers [4]. Even though the enantiomers contain only one chiral center per molecule, the interactions were even larger than those observed between sets of binary equimolar mixtures of diastereoisomers [5,6] which contain two chiral centers per molecule. In addition, NMR frequencies in the sets of diastereoisomers which polarized light in a common direction (either both [+] or both [−]) coalesced in the solid state, but the set which polarized light in opposite directions did not. Thus, in solids spectroscopic properties of enantiomers can be similar to those of diastereoisomers and those of diastereoisomers can be similar to those of enantiomers. A useful presumption is that the conformation of a labile chiral molecule can depend upon the localized chiral environment of the other chiral molecules whether it is structurally an enantiomer or a diastereoisomer. Equimolar D- and L-mixtures do not have a net localized chiral environment because the relative populations of D- and L-isomers are equal.

The spectral properties of an L-isomer surrounded by only other L-isomers would likewise not be fundamentally different from those of a D-isomer surrounded by only D-isomers. However, it is incorrect to conclude that therefore an

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L-isomer surrounded by L-isomers interacts the same as a D-isomer surrounded by L-isomers. Only if the relative populations of L- and D- (*R* and *S* or [+] and [−]) isomers are unequal, is a chiral environment unambiguously defined. Unless there is some D- with the L-isomer, no evidence of an interaction between the two isomers in the sample exists. Since both isomers are present in mixtures of L- and DL-isomers, chiral interactions become experimentally accessible. Vibrational spectroscopy can investigate corresponding chiral interactions of LDLL-mixtures of amino acids in the infrared (IR) frequency range.

Except for threonine and alanine, the NMR spectra of LDLL-mixtures of chiral amino acids are never an average between the L- and the DL-isomer spectra [4]. Binary equimolar mixtures of DL- and L- (i.e. LDLL-) aliphatic structurally different amino acids have also been examined [4]. A difficulty with interpreting the IR spectra of LDLL-mixtures of single amino acids is that although all four isomers have identical chemical structures, two of the three (instead of three of the three) L-isomers can have the same identical frequencies. In contrast, using binary equimolar mixtures of two amino acids potentially simplifies assignment of frequencies to specific chemical structures. In an equimolar mixture of an L-isomer of amino acid (1) and a DL-isomer of amino acid (2), the vibrational frequencies can be assigned to a structure present in only one amino acid. Two sets of data, however, are required for comparison. Each set contains the identical L(1)L(2) composition, but the second half of each set contains the "opposite" composition, L(1)D(2) versus D(1)-L(2). Again the two sets have identical chemical structures, and both contain three L-isomers to every D-isomer. The only difference between the two sets of spectra is the amino acid which contained the D-isomer.

Although mirror images of any conformation of an asymmetrical chemical structure can occur, observations of only symmetrical states such as all L- and all D- or half L- and half D- fail to incorporate data on chiral interactions within a chiral environment. LDLL-mixtures in which half are structurally different amino acids enable comparisons of sites of conformational change among the amino acids within a common corresponding chiral frame of reference. The purpose of this study is to investigate the effects of the chiral and the achiral components on the vibrational frequencies in these mixtures to identify sites and potentially the mechanism of conformational change within mixtures of amino acids related to chiral differences.

EXPERIMENTAL

Samples of L-, DL- and LDLL-isomers of amino acids (Sigma) were prepared as previously reported [4]. The IR spectra were collected on a BioRad Model FTS 65 Fourier transform IR spectrometer by diffuse reflectance using neat samples without further dilution. Particle size was comparable among corresponding samples. Spectra were taken from 4000 to 400 cm^{-1} (data from 1800 to 400 cm^{-1} reported here) at 4 cm^{-1} resolution using KBr as the background standard with 64 scans per spectra. Spectra are shown as taken without baseline adjustment or other manipulation.

VIBRATIONAL SPECTRA

Comparison between the three proline spectra indicated unambiguously that L-proline (Fig. 1c) and LDLL-proline (Fig. 1a) were different, and

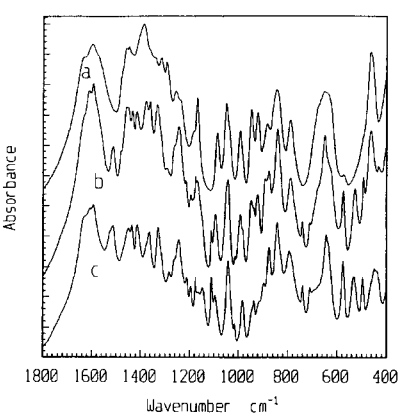

Fig. 1. Diffuse reflectance IR spectrum of proline: (a) LDLL-Pro, (b) DL-Pro, and (c) L-Pro.

further that the IR spectrum of DL-proline (Fig. 1b) was intermediate between them. Thus interactions between chiral components containing an unequal chemical composition (one fourth D-proline with three fourths L-proline) resulted in molecular clusters different from those with equal compositions of chiral components (in DL-proline). In the NMR spectra, DL-proline was also intermediate between L-proline and LDLL-proline [4]. Together, the IR and the NMR results are strong empirical evidence confirming that interactions between mixtures of chiral compounds at asymmetrical mole ratio concentrations can be significantly larger than within equimolar mixtures.

Attempts to explain why spectra of L-proline and DL-proline are different are unsatisfactory unless those same explanations reveal why the spectra of L-proline and LDLL-proline are also dissimilar. Since the frequency differences are independent of chirality (exactly the same aliphatic ring structure, carbonyl and amine occur in each isomer), they must be conformationally, and not structurally related. Analysis of the spectral data must explain why in these molecular clusters one fourth D-proline has a greater effect on the conformation of L-proline then two fourths D-proline. Data manipulation was identical for all sets of data. Therefore, instrumental factors cannot explain the reason for these spectral differences. The particle sizes are also microscopically comparable among sample sets; microcrystallinity differences do not explain these differences. Samples were not diluted, e.g. in KBr, to avoid differences between IR and NMR sample preparation.

Because the same chemical structures existed in each isomer, the changes in vibrational frequencies cannot be assigned to either a D- or an L-isomer. The three vibrational spectra of proline suggested that the conformation of the carbonyl groups was different for the three samples. Some changes in the amide I band region around 1630 cm^{-1} occur [7]. The sharp amide II frequency at 1514 cm^{-1} present in the L-isomer was smaller in the DL- and not apparent in the LDLL-isomer. Similar changes were observed within the frequency range which could be assigned to the amide V and amide VI bands (738 and 1415 cm^{-1}). Corresponding changes also occurred for at least four other sharp peaks (1176, 1168, 948 and 576 cm^{-1}). In the NMR spectra of LDLL-proline [4], two carbonyl frequencies were detected at a ratio of about 4:1. In DL-proline, only the first carbonyl frequency (ratio 1:0) was observed. In L-proline, both frequencies were present, but at a ratio of about 1:4. The simplest conformational explanation for the spectral differences is that the carbonyl groups align differently in molecular clusters containing only L-isomers, than when one in four isomers is a D-isomer.

It is well known that the anisotropy of the benzene ring causes the rings to align perpendicular and not parallel to each other in the solid state [8]. Analogous ordering of the carbonyl groups based on anisotropy may explain the symmetry of the DL-mixture. Most of the carbonyl groups may also be in a similar perpendicular orientation in both the LDLL- and the DL-isomers, but the conformation of the remainder of the chemical structure in the LDLL-mixture would inherently be dissimilar because three L-isomers can never be placed symmetrically into a four-unit cell. Replacing the remaining D-isomer in an LDLL-mixture with an L-isomer would require a major reordering of the molecular cluster. This analysis suggests that the maximum asymmetrical chiral environment in the solid state would always occur at some intermediate mixture of L- and DL-isomers.

The symmetrical and/or asymmetrical packing among LDLL-molecular clusters could be changed by replacing half the composition with structural analogues of proline, i.e. other aliphatic amino acids. The net achiral environment would remain the same if the DL-amino acid is different. The change in the chiral environment can be examined by replacing the L-half instead of the DL-half. Both sets contain equimolar amounts of each structural group and each has three fourth L- and one fourth D-isomers. The only a priori difference discernible is which amino acid contains the D-isomer.

Comparing the vibrational spectra of LDLL-mixtures containing two amino acids, the set containing LDLL-(Ala + Pro) (Fig. 2b) and LLDL-(Ala + Pro) (Fig. 2a) was the most similar. The LDLL-

and LLDL-designations of chemical composition mean the same as DL-Ala + LL-Pro and LL-Ala + DL-Pro, respectively, except that the latter designations incorrectly imply that association between like amino acids predominates over affinity between structurally different amino acids. From 1800 to 400 cm^{-1}, the largest difference between the two spectra was in the carbonyl region from about 1600 to 1500 cm^{-1}. In addition, the LDLL-mixture of Ala + Pro had five frequencies which were absent in the latter mixture (1300, 1085, 985, 789 and 454 cm^{-1}) and correspondingly lacked four frequencies which were present in the latter mixture (1353, 1048, 886 and 462 cm^{-1}). Half of these unassigned frequencies (1086, 1048, 789 and 464 cm^{-1}) were present in the LDLL-mixture of proline. Only one or two frequencies present were identical (within 2 cm^{-1}) for DL-Pro and LLDL-(Ala + Pro). Since few frequencies could unambiguously be assigned to the D-component in L-proline, spectral information was inadequate to determine proline conformational changes. The IR data on alanine, supported by the solid state NMR data [4], indicated that the spectral changes were primarily due to a change in proline and not to a change in alanine.

The vibrational frequencies in the LDLL-mixture of Val + Pro (Fig. 3c) were quite different from those of LLDL-(Val + Pro) (Fig. 3a). Again the predominant differences occurred in the amide carbonyl range of about 1700–1450 cm^{-1}. Between the two sets, eight frequencies in the 2000–400 cm^{-1} range were within 2 cm^{-1} of each other. The frequencies which were in common cannot be related directly to the asymmetry from chiral differences in chemical composition. Of the largest peaks, five of the frequencies in the LDLL-mixture of Val + Pro which were different for the two sets were also within 2 cm^{-1} of frequencies in L-Pro, but 14 were within 2 cm^{-1} of frequencies in DL-Val (Fig. 3b) and eight frequencies were dissimilar to either component. Correspondingly, four of the frequencies in the LLDL-mixture of Val + Pro which were different for the two sets were within 2 cm^{-1} of frequencies in DL-Pro, and nine were within 2 cm^{-1} of frequencies in DL-Val, but 16 frequencies were dissimilar to either component.

These results are consistent with a potentially quite different conformation of proline molecules in the presence of equimolar DL-Val than in the presence of equimolar L-Val. In contrast, the frequencies corresponding to DL-Val were apparently not greatly influenced by the presence of equimolar L-Pro. The conformations of both L-Val and DL-Pro may also be quite different from achiral mixtures of single amino acids. Since the frequencies which change could potentially be assigned to structures in either amino acid, assignment of some frequencies to chemical struc-

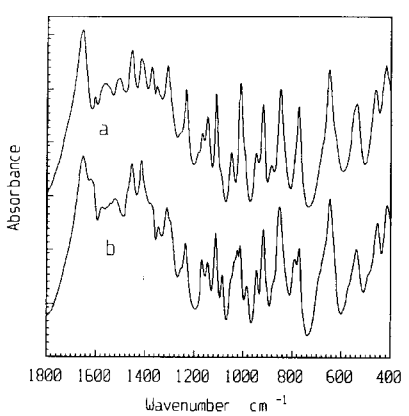

Fig. 2. Diffuse reflectance IR spectrum of equimolar alanine + proline: (a) LL-Ala + DL-Pro (LLDL-), (b) DL-Ala + LL-Pro (LDLL-).

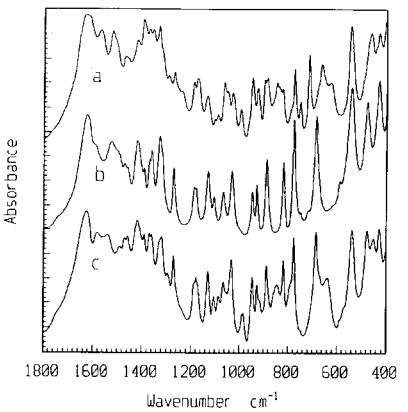

Fig. 3. Diffuse reflectance IR spectrum of equimolar valine + proline: (a) DL-Val + LL-Pro (LDLL-), (b) DL-Val and (c) LL-Val + DL-Pro (LLDL-).

ture could prove to be difficult. Using two different spectroscopic techniques simplifies such assignments. The solid state NMR spectra [4] suggest that the terminal methyl groups of Val may be a site of interaction difference. Isopropyl $C(CH_3)_2$ symmetrical scissors and $C(CH_3)_2$ skeletal vibrations in DL-Val are both split (1370 and 1360 cm^{-1} plus 1186 and 1179 cm^{-1}). In the mixture of L-Pro with DL-Val, the corresponding frequencies are similar to DL-Val alone (1371 and 1361 cm^{-1} plus 1186 and 1178 cm^{-1}), but the intensities of the two skeletal vibrational frequencies are no longer equal (the band at 1186 cm^{-1} is only a slight shoulder compared to that at 1178 cm^{-1}). The same four frequencies are split further apart in the mixture of L-Val with equimolar DL-Pro (1372 and 1356 cm^{-1} versus 1189 and 1173 cm^{-1}). Overall, the spectral differences for the same structural feature was more different between the L- and LDLL-mixtures than between the DL-amino acid alone and the DL-isomer in the mixture.

The differences in vibrational spectra due to chiral interactions between LLDL-(Ser + Pro) (Fig. 4a) and LDLL-(Ser + Pro) (Fig. 4b) were even greater than for the previous mixtures. Only four major frequencies were clearly in common for both sets of data (1470, 1408, 1168 and 667 cm^{-1} were within 2 cm^{-1} of each other). Primary frequencies were present only in LLDL-(Ser + Pro) at 1646, 1496, 1233, 1127, 1013 and 918 cm^{-1} and conversely, primary frequencies were present only in LDLL-(Ser + Pro) at 1678, 1448, 1375, 1318, 1247, 1169, 1039, 982 and 640 cm^{-1}. The frequencies are so different that without knowing the chemical composition of each, one could incorrectly presume that they were even chemically different. Large differences in the conformation between the mixtures were also observed by solid state NMR [4]. The difficulty, however, is not assigning peaks to chemical structures, but assigning them to chiral conformations of chemical structures. The unavoidable fact remains that L- and DL-conformations of amino acids in the solid state may not be the same as their conformation in a different chiral environment. In chiral compounds, specific conformations of functional groups may have characteristic frequencies even in amorphous solids. Both similar and dissimilar conformations within an amino acid in binary equimolar mixtures were detected by diffuse reflectance IR spectroscopy on changing the second L- or DL-amino acid.

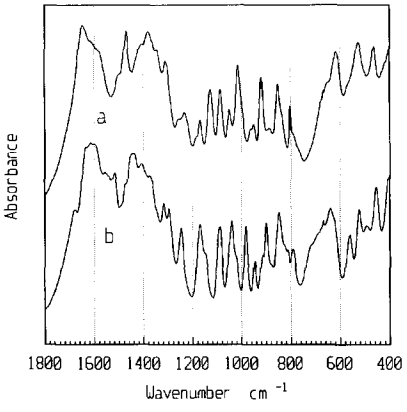

Fig. 4. Diffuse reflectance IR spectrum of equimolar serine + proline: (a) LL-Ser + DL-Pro (LLDL-), (b) DL-Ser + LL-Pro (LDLL-).

DISCUSSION

A phenomenological interpretation of these results is that the interactions among the amino acids in the solid state is diastereoisomeric. The physical, chemical and spectroscopic properties of individual D- and L-isomers are identical because they are enantiomers. In mixtures of unequal proportions of both isomers, the properties of D- and L-enantiomers are quite different compared to those in mixtures containing equal proportions of each. The physical and spectroscopic properties of the asymmetrical molecular clusters cannot be predicted from those of symmetrical molecular clusters of the same components.

The consequence of labeling chiral interactions in a mixture as diastereoisomeric instead of enantiomeric is that common chiral frames of reference (e.g. *R* and *S*) at four sites, not just two, are required to fully characterize that mixture. The properties of mixtures of 1*R*,2*R*-ephedrine and 1*R*,2*S*-ephedrine (in which 1 and 2 refer to a site within the ephedrine molecule)

are not equivalent to those of 1*R*,2*R*-ephedrine and 1*S*,2*R*-ephedrine in the solid state [5,6]. Correspondingly, the properties of mixtures of 1*S*,2*S*-amino acids and 1*S*,2*R*-amino acids (in which 1 and 2 refer to an amino acid within a molecular cluster) are not equivalent, and inherently for the same reason mixtures of 1*S*,2*S*-amino acids and 1*R*,2*S*-amino acids are different. The only structural difference between the sets of *SSRS*-mixtures is that for the diastereoisomeric mixtures, half of the chiral sites are chemically on the same molecule. Interchanging the position of half of the different chiral sites in either case does not result in symmetry.

Site specific ligand binding of a chiral antibiotic to a D,D-alanyl alanine peptide fragment has been reported [9,10]. Chiral ligands also have affinity for the terminal L,D,D sequence [11]. Because of this mixed chiral environment, diastereoisomeric interactions cannot be precluded in its association. The binding of other chiral ligands to only L-peptide sequences could also occur by the same mechanism [12].

Fundamentally, structures interact only via frequencies. The use of the chiral L,D or *R*,*S* designation should therefore be considerably problematic to spectroscopists because no known frequencies can be directly related to *R*, *S*, D or L chemical features. Since there are no known frequencies which can define a configuration as *R* or L, there is no frequency path through which energy between *R*- and *S*- or L- and D-isomers can transfer. The spectroscopic evidence of direction in chirality is observed in the [+] or [−] sign for optical rotation or in the relative phase in a circular dichroism signal, none of which can predict an absolute *R*, *S*, D or L designation. Thus correct labeling of four chiral sites in diastereoisomeric mixtures may in fact be [+] and [−], referring to the phase sensitive optical properties of chiral compounds. L-Pro rotates plane polarized light in a [−] direction whereas L-Val rotates plane polarized light in a [+] direction. (The LDLL- and LLDL-mixtures could also be labeled as [−][+][−][−] and [+][+][+][−].) Specific frequencies related to exactly identical functionalities at matching concentrations appear, disappear, and shift while others remain the same. A partial interpretation is that unique conformations occur in the mixtures. This, however, does not answer the necessary question of whether conformations of chiral mixtures at asymmetrical mole ratios are necessarily different than those of the same components at symmetrical mole ratios.

Quantum mechanical treatments of optical properties of chiral compounds [13,14], in assuming that only two states are necessary to fully define chiral interactions (e.g. D and L, *R* and *S* or [+] and [−]), are inherently unable to correctly predict frequencies and frequency changes in LDLL-mixtures of enantiomers in the solid state. The source of these frequency differences can be the asymmetrical components of these same chiral optical frequencies which happen to coadd to zero in DL-mixtures. Assuming that the symmetrical components of these same frequencies add to zero in the LDLL-mixtures, no frequencies present in any mixture of isomers would not also be present in each constituent isomer.

The frequency and phase of the optical spectroscopy signals can depend upon both a chemical and chiral environment. If in the solid state at asymmetrical concentrations of chiral components the relative signs and phases of corresponding frequencies do not coadd to zero, the net force from chirality on molecules from the same chiral frame of reference would not be zero. Since in LDLL-molecular clusters only half the chiral forces would be equal (with an opposite direction), only the conformations of the DL-half could be accounted for by symmetrical interactions. The effect on conformation from the asymmetrical components (the LL-half) would then be ignored. A rigorous quantum mechanical treatment of chiral interactions must include both the symmetrical and asymmetrical halves. Since the LL-half contains two L-isomers, stoichiometrically its net effect on the LDLL-cluster is to disperse any asymmetry present in an individual L-isomer among twice the number (or volume) of molecules.

Inadequate definition of initial states may be a major potential source in the misinterpretation of chiral interactions. L-isomers are treated as monomers, and racemates of DL-isomers as dimers. If both D- and L-isomers have identical

properties, there is no reason to presume L-isomers form dimers more readily than DL-isomers form dimers. For identical reasons in LDLL-(Val + Pro), there is no inherent reason to predict L-Val and D-Val, or L-Pro and D-Pro form dimers more readily than L-Val and L-Pro, or D-Val and L-Pro. Pairwise affinity among sets could easily be unequal, but there is no structural basis for predicting which sets are more likely to associate randomly or pairwise.

A similar result is observed in matrix algebra. A four-member array containing three identical elements is never mathematically equivalent to the same array in which two elements in any non-identical row (or column) have been interchanged, even though the same pairwise components are present in each set. An unambiguous answer depends upon the absolute configuration (initial position) of the four components in the array.

Because peptides contain at least as many chiral centers as its component amino acids, ignoring asymmetrical interactions among chiral centers could prevent accurate predictions of peptide conformation. Peptide conformation and protein binding in the solid [15] and solution state [16] may involve the same localized "diastereoisomeric" mechanisms which explain the changes in amino acid conformation. Assuming that conformational changes among labile L-amino acids in molecular clusters involve only the symmetrical components of frequencies at chiral centers could seriously bias interpretation of chiral interactions.

Conclusions

It is essential to identify and predict the largest reversible physical and spectroscopic changes which occur on mixing labile stereoisomers. Molecular clusters containing a 3:1 mixture of L- and D-isomer amino acids have vibrational spectra which are not characteristic of either the pure isomers or the equimolar (i.e. DL-) mixtures. The changes in the spectra may result from the asymmetrical conformations within the LDLL-molecular clusters. This same interpretation is self-consistent with solid state NMR results on the same mixtures. Symmetrical conformations in mixtures of labile chiral compounds do not adequately reconstruct asymmetrical conformations which contain the same molecules.

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